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Cellular Immune Functions after Heart Operations

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Eight patients were observed by mitogen stimulation and E-rosetting after heart operations with and without cardiopulmonary bypass. The first sample of blood was taken before surgery, the second on the afternoon of the same day and for a week daily examinations were carried out. A depression of mitogen-stimulated lymphocyte transformation was observed, parallel with a simultaneous T cell depression and a general decrease in the total WBC count. The lowest values were found during the first three days after both open and closed heart operations. After open heart surgery it took a longer time until the values had returned to normal.

In two other cases the lymphocytes were examined for suppressor activity. The suppressive effect exerted on the response to mitogenic and allogeneic stimulation was not uniform. Suppressor activity was more affected by surgery than the helper effect in these two cases.

Immune defense may be affected by many factors including surgery and anaesthesia. The surgeon's wish to keep his patient's condition under control in all possible respects meets here the growing interest in immune functions. A study was therefore performed of the cellular immune response of patients subjected to open heart surgery as compared to changes occurring after closed heart operations. The effect of anaesthesia can be considered similar in both cases, and immunologically the heart patients can be regarded as healthy persons, in contrast to patients with inflammatory or malignant disease.

Patients and Methods

Eight patients after heart operations with and without cardiopulmonary bypass were followed by mitogen stimulation and E-rosetting. The first sample of blood was taken on the morning before the operation, the second the same afternoon just after surgery. Daily examinations were carried out for a week and later the tests were repeated occasionally for six weeks.

The cellular studies did not separately take into account the differences between the patient's own blood and the don blood transfused during surgery.

Lymphocyte separation and standard culture methods

These had been described previously in detail [11]. Briefly, phytohaemagglutinin (PHA, Difco) was used for assessing the mitotic capacity of peripheral blood lymphocytes isolated over Ficoll-Uromiro gradient. The optimum concentration was established in each individual case. Cultures were labelled with 1 μCi of 3 H-thymidine on day 3 for 5 hrs and harvested for scintillation spectrometry. All cultures were set up in triplicates. Results were expressed as dpm/10^6 cells.

For investigation of T suppressor cells, cryopreserved lymphocytes were used. The method has been described previously [11].

E-rosette (ER) formation

Sheep red blood cells and lymphocytes were mixed in a ratio of 40 : 1 in Parker 199 medium supplemented by 8—9% absorbed fetal calf serum, incubated at 37°C for 15 min, centrifuged at 50 g for 15 min and left at 4°C overnight. A total of 200 lymphocytes were counted and all lymphocytes binding more than three SRBC were taken as E rosettes.

Induction of T suppressor cells

Responder donor lymphocytes (5×10^6 ml) were cultures with Con A (50 μg/ml) in a humidified 5% CO_2 incubator for 48 h, then the cells were washed and resuspended in RPMI containing 10% human AB serum at a concentration of 1×10^6 lymphocytes/ml *(R-ConA)*.

Aliquots of these cells were subsequently treated with mitomycin C (25 μg/ml) at 37°C for 30 min, and washed three times *(R-ConA-M)*.

Suppression of the response to mitogens

100 μg of 10^6 responder cells (R) were cultured with mitogen (Con A or PHA) on microtest plates (Greiner). All assays consisted of responder cells + mitogen with or without Con-A induced suppressor cells (R-Con-A). Cultures were incubated at 5% CO_2 tension and 37°C for 3 days.

Suppression of the allogeneic reaction

For mitomycin C-treated stimulator cells *(SM)* we used pooled standard cells of three donors with different HLA-ABC and -DR types. Aliquots of responder *(R)* and mitomycin C-treated stimulator cells *(SM)* were co-cultured with an equal number (1×10^5) of the Con A pretreated *(R-Con-A)* lymphocytes. The assay protocol consisted of responder cells plus allogeneic stimulator

cells with and without Con-A induced suppressor cells, or mitomycin C-treated Con-A blast cells. All experiments were performed in quadruplicate. The per cent of suppression was calculated in the following way:

$$\text{S index } \% = \frac{1 - (R+SM+R-ConA) - (R+RM+R-ConAM}{(R+RM) - (R+M)} \text{ cpm} \times 100 .$$

Results

A depression of *PHA-stimulated lymphocyte transformation* went parallel with T cell depression, wich latter was associated with a general decrease in the total WBC count (Fig. 1.). The lowest values were found during the first

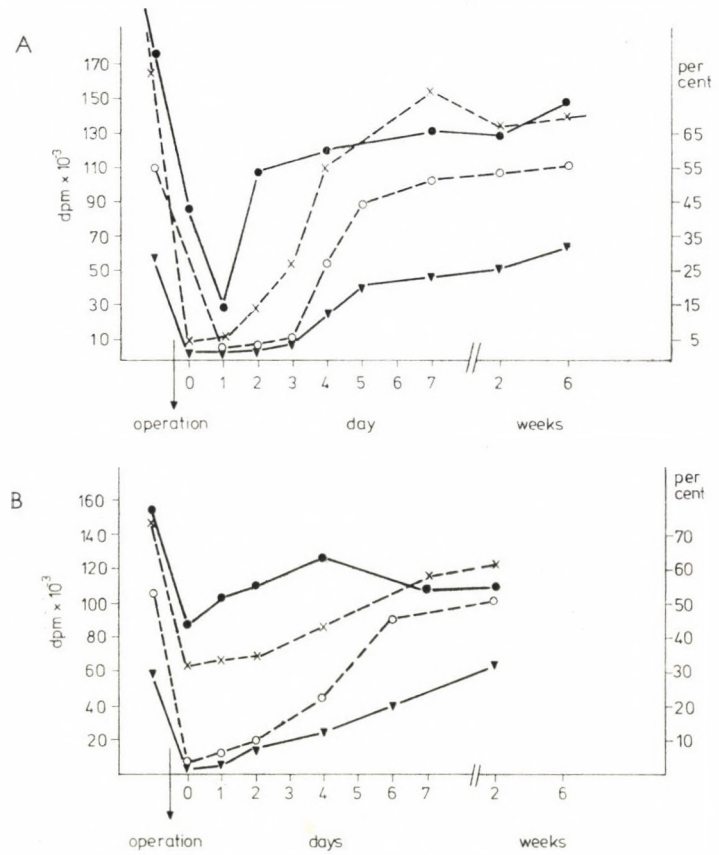

FIG. 1. PHA stimulation and E rosette formation of a heart patient with (A) and another without (B) cardiopulmonary bypass.
▼: number of lymphocytes, per cent
○: ERFC, per cent
●: lymphocyte transformation in pooled AB serum (dpm/10^6 cells)
×: lymphocyte transformation in autologous serum (dmp/10^6 cells)

three days after open heart cases and closed cardiac operations as well. After open heart operations it took a longer time until the values had returned to normal.

The depression of the PHA response was greater if instead of normal pooled AB serum the patient's own plasma was added to the cultures (Fig. 1).

In Fig. 2 cases of some other heart patients are shown, 5 with and 3 without cardiopulmonary bypass, followed up for 8—21 days.

Suppressor cell activity was tested in two cases: one day prior to operation and at four days after surgery. One of the two patients (K. A. in Table I) showed results quite similar to those found in normal individuals, while the other (S. L. in Table I) presented a lesser initial response to Con A and in MLC.

A 52—73 suppressor activity was found in the first case one day after operation then it disappeared for two days only to reappear by the fourth day.

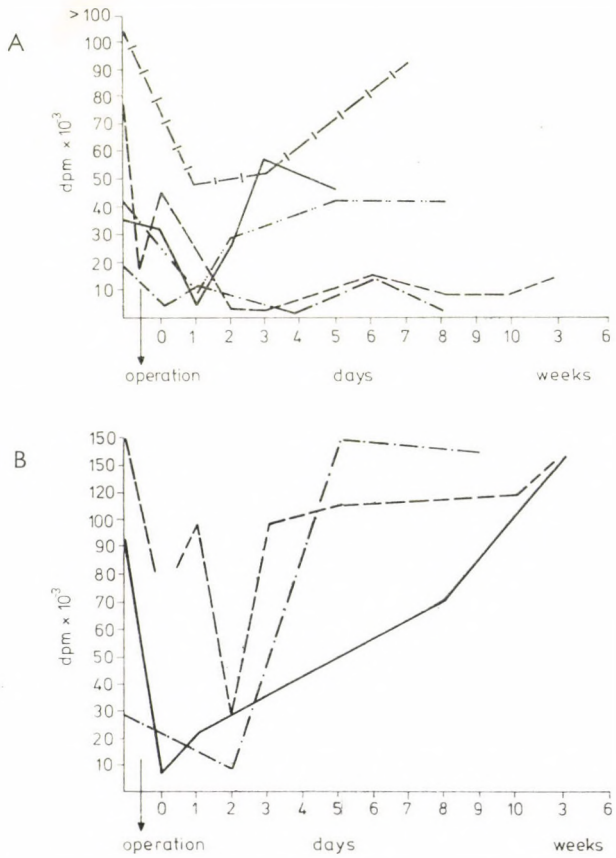

FIG. 2. PHA stimulation of 8 cardiac patients. Five patients with cardiopulmonary bypass (A) and another three patients without cardiopulmonary bypass (B)

TABLE I

*Representative values of Con A, PHA and allogeneic response with and without T suppressor cells added**

	Con A effect (cpm)	Suppr. values (cpm)	Suppression per cent	PHA effect (cpm)	Suppr. values (cpm)	Suppression per cent	MLC effect (cpm)	Suppr. values (cpm)	Suppression per cent
Normal control	18 936	10 963	43	24 837	9 321	63	5 680	3 864	42
K. A. 0	22 480	10 258	55	3 009	841	73	4 565	2 232	52
K. A. 2	304	1 296	<1	927	219	77	175	218	<1
K. A. 3	380	7 782	≪1	1 761	10 044	<1	90	59	35
K. A. 5	1 235	3 770	<1	6 621	16 324	<1	86	61	31
S. L. 0	1 647	6 943	<1	3 403	5 272	<1	1 561	407	74
S. L. 3	6 201	5 722	8	17 598	17 524	1	530	610	<1
S. L. 5	459	531	<1	12 390	10 130	19	1 670	395	77

The values are before (0) or after (2, 3, 5 days) surgery
* Average cpm of quadruplicate counts

The changes in the suppressive effect on the allogeneic response were less dramatic in this case but went parallel to the mitogen-induced response.

The second patient (S. L.) who showed a weaker initial response and no suppressor activity after mitogen stimulation before surgery, seemed to react by the 2nd day after surgery. The same patient exhibited quite similar changes in the MLC test using pooled allogeneic lymphocytes.

Discussion

Heart operations in immunologically healthy persons offer a good model to study the effects on the immune system of surgical trauma and anaesthesia. This is the reason why in most of the few reports on the subject, heart surgery patients were included.

LUNDSTROM et al. [7] reported that the in vitro phagocytosis of *Staphylococcus albus* by polymorphonuclear leukocytes was depressed to 20% of the normal immediately after cardiopulmonary bypass. The reduced phagocytic capacity persisted for 18 days. Kaplan et al. [8], however found normal phagocytic and bactericidal capacity of the polymorphonuclear leucocytes in 11 patients who had undergone open heart surgery with cardiopulmonary bypass.

Some clinical studies revealed quantitative changes in serum immunoglobulins and complement following injury and operation [5], especially after cardiopulmonary bypass [9].

After minor surgery performed under general anaesthesia e.g. for benign breast disease, VOSE and MOUDGIL [14] found no significant fall in PHA-responsiveness on the 1st postoperative day. They found, however, a significantly diminished capacity to induce target cell lysis in ADCC, as compared to the capacity of the same patients.

The lymphocyte transformation test has frequently been performed during and after heart operations and in follow-up studies PARK et al. [8], RIEDLE and BERENBAUM [12] found decreased PHA-responsiveness of peripheral blood lymphocytes in patients who had undergone extensive operations. PHA responsiveness was most depressed in patients with cancer and those subject to heart operations. Maximum depression occurred one to eight days after the operation. This interval is similar as in our cases and is in contrast to the data of HAN [4] and COCHRAN et al. [1] who found that the response to PHA of operated patients was depressed up to 3 weeks after surgery.

SLADE et al. [13] studied a number of parameters of cell-mediated immunity in 12 healthy kidney transplant donors. Total blood lymphocyte count, B cell count, T cell count, the mitogen response and MLG reactivity decreased after the induction of anaesthesia and continued to decrease during nephrectomy. Their findings indicate that anaesthesia by itself may cause a depression in these functions. Their late results were identical with those of ours, insofar as all in vitro tests were normal by the fifth postoperative day.

These data and our own observations show that the period of postoperative depression of cellular immune functions is limited to a few days and represents apparently a small degree of depression instead of a complete unresponsiveness.

According to our first examinations shown in Table I, changes in the T cell suppressor activity are also limited to the first few postoperative days. In our first patient (K. A.) suppressor activity showed some impairment, similar to other immune functions studied postoperatively. In the second case (S. L.) there was no detectable suppressor activity in the ConA- and PHA-response before operation, possibly as a consequence of helper cell predominance. In this case, however, this suspected helper cell activity was already reduced on the second postoperative day, as revealed by the detectable suppressor activity.

In the second patient's case, the contradiction between the suppressor T cell activity in response to mitogens and to allogenic stimulation may be explained by the differences in the participating cell populations in the two reactions. This divergence was repeatedly recognized during the HLA—DR Intertransplant Workshop held in 1979: the results of allogeneic reactivity corresponded only partly to those observed in regard of PHA-reactivity [10].

Our two examples seem to show that the generally observed postoperative depression of lymphocyte function should not be attributed to some suppressor

T-cell-mediated mechanism. Apparently, there might be differences in the helper and suppressor cell activities in any individual case. Any alteration in the balance of stimulation and suppression may influence the actual values of the immune function tests. In experimental models the stimulatory activity seems to be much more resistant than the suppressor function [3]. Testing of these functions will allow a deeper insight into these regulatory mechanisms during the early postoperative period. It seems, anyway, that after a transitory decrease lasting for a few days, immune reactions return to normal.

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Gestaltung der zellulären Immunfunktionen nach Herzoperationen

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Bei 8, eine offene oder geschlossene Herzoperation überstandenen Patienten wurden postoperativ die Gestaltung der mitogen-induzierten Lymphozytentransformation und die der Rosettenzahl untersucht. Die erste Blutprobe wurde präoperativ, die zweite unmittelbar nach dem Eingriff und die darauffolgenden eine Woche lang täglich entnommen.

Die Verringerung der mitogen-induzierten Blasttransformation zeigte, sowohl nach offenen als auch nach geschlossenen Herzoperationen, eine Parallelität mit der Abnahme der T-Zellzahl und der Gesamt-Leukozytenzahl. Laut der Untersuchungen wurden durch offene Operationen länger haltende Depressionen herbeigeführt.

Bei weiteren zwei Patienten wurde auch die Gestaltung der suppressorzelligen Aktivität analysiert. Die mit Con-A aktivierte supressorzellige Aktivität zeigte — selbst im Laufe der Kontrolle–Abweichungen in den mitogenen und allogenen Reaktionen. Die Untersuchungen sprachen dafür, daß die Supressorwirkung während der Operation empfindlicher auf die Änderungen reagiert als die Helper-Aktivität.

Формирование клеточных иммунологических функций после операций на сердце

И. ПЕТРИ, Г. КАЙЗЕР, Т. ГААЛ, Г. КОВАЧ и Г. ПЕТРИ

У восьми больных, которые перенесли открытую или закрытую операцию на сердце, мы изучали митогенно-индуцированную трансформацию лимфоцитов и розеткообразование в после операционный период. Первую пробу крови брали певед операцией, вторую непосредственно после операции, затем пробы брались ежедневно в течение недели.

Снижение митогенно-индуцированной бластозной трансформации обнаружило параллелизм с уменьшением числа Т клеток и общего количества белых кровяных клеток, как у больных, перенесших закрытую, так и у перенесших открытую операцию на сердце. Наши исследования указывают на то, что открытая операция вызывает более продолжительную депрессию.

Кроме того, у двух больных мы изучали также супрессорно-клеточную активность. Активированная Со А супрессор-клеточная активность показала отклонение в митогенных и аллогенных реакциях и по ходу следования. Результаты наших исследований показывают, что супрессорное действие по ходу операции более чувствительно к изменениям, чем стимулирующее.

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